Within-host evolution of Pseudomonas aeruginosa toward iron acquisition from hemoglobin in polymicrobial CF infections

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can be ported into systems-biology platforms, which will have wide utility in microbiology research.

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MICROBIAL INTERACTIONS AND EVOLUTION IN CHRONIC CYSTIC FIBROSIS INFECTIONS

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Chronic cystic fibrosis (CF) airway infections provide opportunities for fundamental investigations related to microbial evolutionary dynamics, diversity, and interactions within a natural polymicrobial ecosystem. Patients with CF are predisposed to airway infections from a wide range of microbial species of which Pseudomonas aeruginosa is the major contributor to patient morbidity and mortality. It is well-established that diverse factors such as the host defense, antibiotic treatment in the clinic, and a heterogeneous distribution of nutrients drive P. aeruginosa evolutionary adaptation to the lung environment. However, whether interactions with other infecting microbes is also an evolutionary driver is not well understood. To begin to explore the relationship between evolution and microbial interactions, we have focused on two distinct P. aeruginosa lineages (called "DK1" and "DK2") that have transmitted among and evolved in CF patients in Denmark during the last 40 years. Although most patients are infected with either clone-type, DK1 and DK2 have also been co-existing in many patients for extended periods. Here, we focused on a single patient with a mixed infection containing both clone types. To investigate interactions between DK1 and DK2 we sequenced the genomes of isolates sampled from the patient over 15 years. Surprisingly, we identified isolates with mosaic genomes: These isolates (which we call "DK1/2") had DK2-based genomes but containing regions of DK1 DNA acquired by horizontal gene transfer and recombination. DK2 isolates are sensitive towards R5 pyocins produced by other P. aeruginosa lineages. We show that the transferred regions provide enhanced R5 pyocin resistance to DK1/2. Our data suggest that the within-host genetic interactions between co-infecting DK1 and DK2 strains could be driven by super-infections with R5 producing genotypes. To more systematically explore interactions between DK1 and DK2, we are mapping phenotypic interactions between 100 DK1 and DK2 isolates sampled from multiple patients. For that purpose we are currently performing a pairwise screening on agar surfaces using differentially fluorescent-tagged strains to assess neutral, negative or positive effect. Our results point towards an unexplored area for novel interference treatment strategies in relation to microbe-microbe interactions.

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WITHIN-HOST EVOLUTION OF PSEUDOMONAS AERUGINOSA TOWARD IRON ACQUISITION FROM HEMOGLOBIN IN POLYMICROBIAL CF INFECTIONS

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Bacterial pathogens require iron to survive and colonize a human host but their access to free iron is often limited by iron-withholding process where free iron is bound by proteins such as hemoglobin. Although most pathogens have developed tactics to acquire iron from host proteins, little is known about how evolutionary processes modulate bacterial iron acquisition systems in chronic, polymicrobial infections where interspecies competition for limited iron could be an evolutionary driver. To begin to address this issue, we use chronic airway infections in patients with cystic fibrosis (CF) as a model to investigate evolutionary adaptation to an iron-limited environment in a polymicrobial context. Here, we investigate the within-host evolution of the transmissible P. aeruginosa DK2 lineage sampled from (CF) airway infections over a period of

several decades. We find a positive selection for promoter mutations in P. aeruginosa DK2 leading to increased expression of the phu (Pseudomonas heme utilization) system. By mimicking conditions of the CF airways in vitro, we experimentally demonstrate that increased expression of phuR confers a growth advantage in the presence of hemoglobin, thus suggesting that P. aeruginosa evolves towards iron acquisition from hemoglobin. We also find similar adaptive mutations in the genomes of two additional P. aeruginosa lineages ruling out the specificity of these mutations to this particular lineage. Furthermore, in all three lineages phuR promoter mutations coincide with the loss of pyoverdine production, suggesting that within-host adaptation towards heme utilization is coupled to the loss of pyoverdine production. We hypothesize that this particular adaptation in P. aeruginosa DK2 has an impact on interspecies interaction with other members of the CF polymicrobial community capable of heme utilization. We are currently testing this hypothesis by exploring competition for iron from hemoglobin between P. aeruginosa DK2 and Staphylococcus aureus that are frequently co-isolated from CF infections.

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INNATE IMMUNE DYSFUNCTION IS ASSOCIATED WITH ELEVATED A20 EXPRESSION DURING INFLUENZA A VIRUS/S. PNEUMONIAE COINFECTION IN MICE

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Both, influenza A virus and S. pneumoniae are a leading cause of morbidity and mortality worldwide. However, most influenza-related mortality is not due to the viral infection alone but rather secondary bacterial infections, mainly caused by S. pneumoniae. The mechanisms driving virulent influenza coinfection are poorly defined, making it difficult to develop effective therapeutic strategies. This study investigates signaling events evoked by influenza infections affecting the innate immune response and cellular clearance mechanisms in the lung. Using an in vivo model of subsequent infections with influenza A virus and S. pneumoniae via the intratracheal infection route we show that sublethal influenza infections clearly predispose for severe pneumococcal infections even at low bacterial doses. Coinfected C57BL/6 mice are more susceptible to pneumococcal infection than single-infected mice, resulting in drastically less survival and earlier development of pneumonia and bacteremia. Despite an upregulation of the endogenous TLR inhibitor A20 at 24 hours post secondary infection in lungs of coinfected mice, cytokine analyses show a significant increase of proinflammatory cytokines and increased numbers of neutrophils in the airways of co-infected compared to single-infected mice. This enhanced inflammation associated with tissue damage contributes to the severity of secondary bacterial pneumonia suggesting that influenza-induced A20 is either not sufficient to suppress the inflammation or is dysfunctional.

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REGULATORY CHARACTERISTICS OF VIBRIO VULNIFICUS GBPA, ENCODING N-ACETYL GLUCOSAMINE BINDING PROTEIN AND ESSENTIAL FOR PATHOGENESIS

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Mucin glycoprotein is a major component of mucus layer that is the major site of entry for most pathogens and serves as the initiation surfaces for host-microbe interactions. Vibrio vulnificus, a model human enteric pathogen, is